IGF-1 (Mouse/Rat) ELISA

For the quantitative determination of IGF-1 in serum or plasma (Heparin, EDTA or Citrate) of mice and rats.

For Research Use Only. Not For Use In Diagnostic Procedures.

Catalog Number: 22-IG1MS-E01
Size: 96 wells
Version: 06.12.2022 - ALPCO 2.1

1. Intended Use

The IGF-1 (Mouse/Rat) ELISA is designed for the measurement of insulin-like growth factor-1 (IGF-I) in the serum and plasma (heparin, EDTA or citrate) of mice and rats. For research use only. Not for use in diagnostic procedures.

2. Introduction

In addition to different cell culture models and studies with human material, mice and rats are suitable model systems for basic research and pre-clinical studies. Thus, this ELISA was developed as a tool for IGF-I measurements in mice and rat for use in research and pre-clinical studies. The comparability of IGF-1 in mice and humans is limited, however, some background information on the *human* IGF-I system follows:

Insulin-like growth factors (IGF) I and II play a pivotal role in regulating the proliferation and differentiation of many cell types (1-3). IGF-I is identical to Somatomedin C (Sm-C) (4) and has a molecular weight of 7649 daltons (5). Its major regulators are growth hormone (GH) and nutrition (6). In contrast to many other peptide hormones, IGFs are avidly bound to specific binding proteins (IGFBP). The seven IGFBPs which are known at present (7,8,22) either bind IGF-I and IGF-II with similar affinities or show a preference for IGF-II (9,10).

A major problem of IGF-I measurement results from the interference of IGFBPs in the assay. Direct determinations in untreated serum samples (11) give false values because of the extremely slow dissociation of the IGF-I/IGFBP-3 complexes during the assay incubation (see Figure 1).

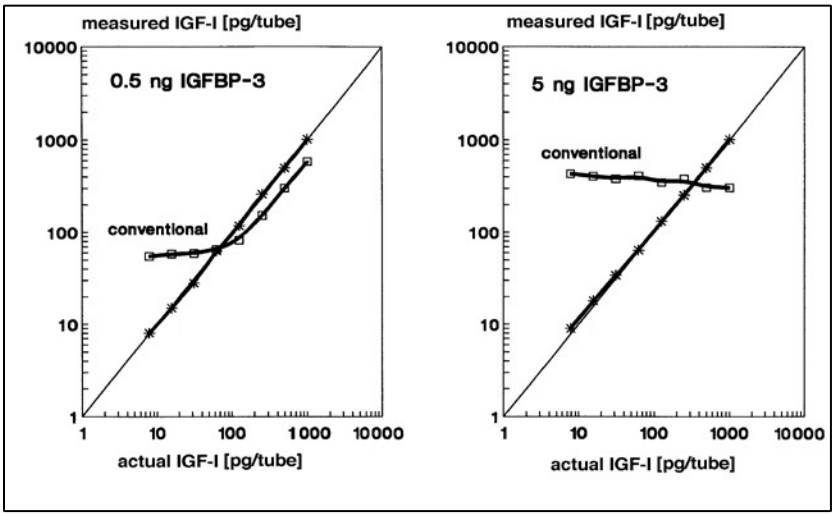

Figure 1: Interference of IGFBP in IGF-I measurements. Known concentrations of IGF-I were assayed in the presence of 0.5 ng (left) or 5 ng (right) hIGFBP-3 by a conventional (□) and by the IGFBP-blocked assay (*).

To avoid these difficulties, this assay was developed in which special sample preparation is not required before measurement, except for the acidification and dilution of the sample in a specially designed buffer system.

3. Principle of the Assay

The IGF-1 (Mouse/Rat) ELISA is a sandwich assay. It utilizes two specific high affinity antibodies for this protein. The IGF-I in the sample binds to the first antibody which is immobilized on the microtiter plate. The biotinylated second specific anti-IGF-I antibody binds in turn to the immobilized IGF-I. Streptavidin-conjugated peroxidase is added and in the closing substrate reaction, the amount of color generated will be specific and vary quantitatively depending on the IGF-I level of the samples.

In order to dissociate IGF-I from the IGFBPs, the samples must be diluted in an acidic buffer (Figure 2). The diluted samples are then pipetted into the microtiter wells, thus neutralizing the acidic pH. After neutralization of the samples, excess IGF-II occupies the IGF-binding sites of the binding proteins, thus allowing the measurement of the resulting free IGF-I. With this method, the IGFBPs are not removed, but their function and therefore their interference in the assay is neutralized. Due to the extremely low cross-reactivity of the IGF-I antibody with IGF-II, the excess IGF-II does not interfere with the antibody's interaction with IGF-I. The test is performed like a conventional ELISA using a streptavidin-peroxidase enzyme conjugate.

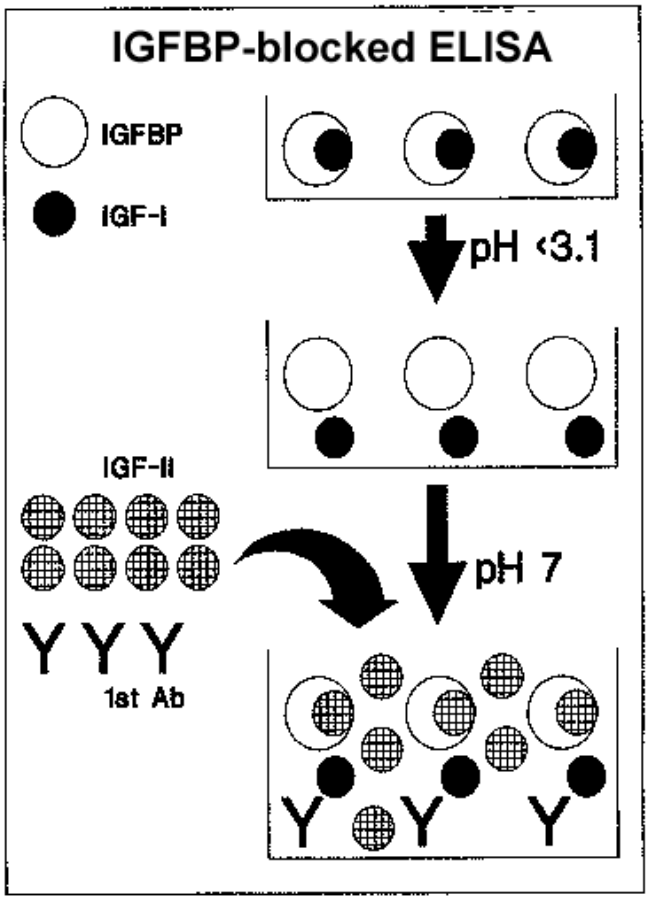

Figure 2: Principle of the IGFBP blocked IGF-I ELISA

4. Warnings and Precautions

For research use only. For professional use only.

This ELISA is intended for research use only and is not for internal use in humans and animals. Strictly follow the test protocol. Use the valid version of the package insert provided with the kit. Be sure that everything has been understood. ALPCO will not be held responsible for any loss or damage (except as required by statute) however caused, arising out of noncompliance with the instructions provided.

Do not use obviously damaged or microbially contaminated or spilled material.

Caution: This kit contains material of human and/or animal origin. Therefore, all components and samples should be treated as potentially infectious.

Appropriate precautions and good laboratory practices must be used in the storage, handling and disposal of the kit reagents. The disposal of the kit components must be made according to the local regulations. A Safety Data Sheet is available on request.

Mouse / Rat Serum
The following components contain Mouse or Rat serum: Control Serum KS1 and KS2.

Reagents A-E, AK, EK, VP, WP
Contain as preservative a mixture of **5-chloro-2-methyl-4-isothiazolin-3-one and 2-methyl-4-isothiazolin-3-one** (<0.015%)

H317	May cause an allergic skin reaction
P280	Wear protective gloves/ protective clothing/ eye protection/ face protection
P272	Contaminated work clothing should not be allowed out of the workplace
P261	Avoid breathing dust/ fume/ gas/ mist/ vapors/ spray
P333, P313	If skin irritation or rash occurs: Get medical advice/ attention
P302, P352	IF ON SKIN: Wash with plenty of soap and water
P501	Dispose of contents/ container in accordance with local/ regional/ national/ international regulations

Substrate Solution (S)
TMB-Substrate (S) contains 3,3´,5,5´-Tetramethylbenzidine (<0.05%)

H315	Causes skin irritation
H319	Causes serious eye irritation
H335	May cause respiratory irritation
P261	Avoid breathing dust/ fume/ gas/ mist/ vapors/ spray
P305, P351, P338	IF IN EYES: Rinse with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.

Stop Solution (SL)

The Stop solution contains 0.2 M acid sulfuric acid (H_2SO_4)

H290	May be corrosive to metals
H314	Causes severe skin burns and eye damage
P280	Wear protective gloves/ protective clothing/ eye protection/ face protection
P301, P330,	IF SWALLOWED: rinse mouth
P331	Do NOT induce vomiting
P305, P351,	IF IN EYES: Rinse with water for several minutes
P338	Remove contact lenses, if present and easy to do. Continue rinsing.
P309, P310	IF exposed or if you feel unwell: Immediately call a POISON CENTER or doctor/physician.

4.1. General first aid procedures:

- Skin contact: Wash affected area; rinse immediately with plenty of water for at least 15 minutes. Remove contaminated clothes and shoes.
- Eye contact: In case of contact with eyes, rinse immediately with plenty of water for at least 15 minutes. In order to ensure efficient rinsing, spread the eyelids.
- Ingestion: After swallowing the product, if the affected person is conscious, rinse out the mouth with plenty of water; seek medical advice immediately.

5. Samples Handling

5.1. Sample Types

Serum samples as well as heparin, EDTA and citrated plasma samples are acceptable. Possible dilution of the sample by the anticoagulant must be taken into account.

Influence of heparin (30 IU/mL), EDTA (6.8 mM) and NaCitrate (0.015 M) on the measurement of IGF-I has been investigated in recovery experiments. Buffer solution was spiked with recombinant IGF-I and the above mentioned substances. No significant influence on the recovery of IGF-I was detected; on average the recovery of recombinant material in comparison to enriched PBS was 108%.

Cell culture medium is suitable as sample matrix after predilution of 1:2 with Sample Buffer PP.

5.2. Sample Collection

Use standard venipuncture for blood sampling. Hemolyzed samples are to be avoided.

Required sample volume: recommended 10 µL, minimum 5 µL

5.3. Sample Stability

In firmly closed sample vials:

- Storage at 20 - 25°C: max. 2 days
- Storage at -20° C: max. 2 years
- Freeze-thaw cycles: max. 2

The storage of samples over a period of 2 years at -20°C showed no influence on the results. Therefore, it is recommended to keep samples refrigerated or frozen as soon as possible after separation of serum or plasma from the red cells and to avoid more than 2 freeze-thaw cycles.

5.4. Sample Preparation: Dilution

- Dilution: **1:100** with **Sample Buffer PP**
- Pipette **990 µL Sample Buffer PP** in PE-/PP-Tube (application of a repeater pipette is recommended in larger series); add **10 µL sample** (dilution 1:100). After mixing use 50 µL of this dilution in the assay.
- Attention: serum and plasma samples must be diluted at least 1:10 in **Sample Buffer PP** to achieve sufficient acidification of the samples.
- Depending on the expected IGF-I values the samples can be diluted higher in **Sample Buffer PP**.

6. Materials

6.1. Reagents Provided

The reagents listed below are sufficient for 96 wells including the standard curve:

MTP	**Microtiter plate** ready for use, coated with hamster-anti-m/r-IGF-I-antibody. Wells are separable.	**8 x 12 well**
A-E	**Standards** lyophilized, (recombinant IGF-I), The concentrations are given on vial labels and on quality certificate.	**5 x 1 mL**
PP	**Sample buffer** ready for use	**1 x 125 mL**
KS1 **KS2**	**Control Sera KS1 & KS2**, lyophilized, (Mouse/Rat Serum), The concentration of each control is given on quality certificate in ng/mL.	**1 x 500 µL each**
AK	**Antibody Conjugate** ready for use, contains biotinylated goat anti-m/rIGF-I antibody**.**	**1 x 7 mL**
EK	**Enzyme Conjugate** ready for use, contains horseradish-peroxidase labelled streptavidin	**1 x 12 mL**
WP	**Wash Buffer** 20-fold concentrated solution	**1 x 50 mL**
S	**Substrate** ready for use, horseradish-peroxidase-(HRP) substrate, stabilized tetramethylbenzidine.	**1 x 12 mL**
SL	**Stop Solution** ready for use, 0.2 M sulfuric acid.	**1 x 12 mL**
-	**Sealing tape** for covering the microtiter plate	**2**

6.2. Materials Required but not provided

- Distilled or deionized water for dilution of the Washing Buffer WP**, 950 mL.**
- Precision pipettes and multichannel pipettes with disposable plastic tips
- Vortex-mixer
- Microtiter plate shaker (350 rpm)
- Microtiter plate washer (recommended)
- Microplate reader ("ELISA-Reader") with filter for 450 and ≥ 590 nm
- Polyethylene PE/Polypropylene PP tubes for dilution of samples
- Timer

7. Technical Notes

Storage Conditions
Store the kit at 2-8°C after receipt until its expiry date. The lyophilized reagents should be stored at –20 °C after reconstitution. Avoid repeated thawing and freezing.

Storage Life

Store the unused strips and microtiter wells airtight together with the desiccant at 2-8°C in the clip-lock bag, use in the frame provided. The reconstituted components, Standards A-E and Control Sera KS1 and KS2 must be stored at -20°C (max. 2 months).
Attention: Standards should be thawed only once. Where required, store aliquoted in adequate volumes. For further use, thaw quickly but gently (avoid temperature increase above room temperature and avoid excessive vortexing). One freeze-thaw cycle showed no significant effect on the test.

The 1:20 diluted Wash Buffer WP is stable for 4 weeks at 2-8°C.

Preparation of reagents

Bring all reagents to room temperature (20 - 25°C) before use. Possible precipitates in the buffers must be dissolved before use by mixing and/or warming. Reagents with different lot numbers cannot be mixed.

Reconstitution

The Standards **A – E** and Control **KS1 and KS2** are reconstituted with the Sample Buffer **PP**. It is recommended to keep reconstituted reagents at room temperature for 15 minutes and then mix them thoroughly but gently (no foam should result) with a vortex mixer.

Dilution

After reconstitution dilute the Control **KS1** and **KS2** with the Sample Buffer **PP** in the same ratio as the sample.

The required volume of Washing Buffer **WP** is prepared by 1:20 dilution of the provided 20-fold concentrate with distilled water.**Assay Procedure**

When performing the assay, Blank, Standards **A-E**, Controls **KS1 and KS2** and the samples should be pipetted as quickly as possible (e.g. < 15 minutes). To avoid variations in results due to differences in incubation times, Antibody Conjugate **AK** and the Enzyme Conjugate **EK** as well as the succeeding Substrate Solution **S** should be added to the plate in the same order and in the same time interval as the samples. Stop Solution **SL** should be added to the plate in the same order as Substrate Solution **S**. All determinations (Blank, Standards **A-E**, Control **KS1** and **KS2** and samples) should be assayed in duplicate. For optimal results, accurate pipetting and adherence to the protocol are recommended.

Incubation

Incubation at room temperature means: 20-25°C.

The Substrate Solution **S**, stabilized tetramethylbenzidine, is photosensitive; store and perform incubation in the dark.

Shaking

The incubation steps should be performed using a suitable microtiter plate shaker. The recommended setting for the plate shaker is 350 rpm. Due to certain technical differences in plate shakers, the rotation speed may need to be adjusted. Insufficient shaking may lead to inadequate mixing of the solutions and thereby to low optical densities, high variation and/or false values; excessive shaking may result in high optical densities and/or false values.

Washing

Proper washing is of basic importance for the reliable and precise performance of the assay. Incomplete washing is common and will adversely affect the test outcome. Possible consequences may be uncontrolled, nonspecific variations of measured optical densities, potentially leading to false result calculations for the examined samples. Effects like high background values or high CVs may indicate washing problems.

All washing must be performed with the provided wash buffer diluted to the 1X working concentration. Washing volume per washing cycle and well must be at least 300 µL.

The danger of handling potentially infectious material must also be considered.

Manual washing should be performed. Wash Buffer may be dispensed via a multi-stepper device, a multichannel pipette, or a squirt bottle. Decant contents into a biohazard bin, then blot plate on absorbent tissue. Wash the plate by adding 300 µL 1X working Washing Buffer WP/well, then decant and blot on absorbent tissue. Repeat this step 4 more times for total of 5 washes.

8. Assay Procedure

Preparation of reagents		Reconstitution:	Dilution
A-E	**Standards**	in **1 mL** Sample Buffer **PP**	-
KS1	**Control Serum 1**	in **500 µL** Sample Buffer **PP**	**1:100** with Sample Buffer **PP**
KS2	**Control Serum 2**	in **500 µL** Sample Buffer **PP**	**1:100** with Sample Buffer **PP**
WP	**Wash Buffer**	-	**1:20** with **distiled water**
Dilute samples 1:100 in Sample Buffer PP, mix immediately, incubate at least 15 minutes, maximum of 2 hours. Use **50 µL** for each well in the assay.			
Before assay procedure bring all reagents to room temperature **20-25°C**.			

Assay Procedure (using duplicates)		
Pipette	**Reagents**	**Position**
50 µL	Antibody Conjugate **AK**	in **all** wells used
50 µL	Sample Buffer **PP** (Blank)	A1/A2
50 µL	Standard **A** (**0.5 ng/mL**)	B1/B2
50 µL	Standard **B (2.5 ng/mL)**	C1/C2
50 µL	Standard **C (6 ng/mL)**	D1/D2
50 µL	Standard **D** (**12 ng/mL)**	E1/E2
50 µL	Standard **E (18 ng/mL)**	F1/F2
50 µL	Control Serum **KS1** (1:100 diluted)	G1/G2
50 µL	Control Serum **KS2** (1:100 diluted)	H1/H2
50 µL	Sample (1:100 diluted)	in the rest of the wells per the plate map
Cover the wells with the sealing tape.		
Sample-Incubation: 1 hour at 20-25°C, 350 rpm		
5x 300 µL	Decant the contents of the wells and **wash** 5x with 300 µL of 1X working Washing Buffer **WP**	In each well
100 µL	Enzyme Conjugate **EK**	In each well
Cover the wells with the sealing tape.		
Incubation: 30 minutes at 20-25°C, 350 rpm		
5x 300 µL	Decant the contents of the wells and **wash** 5 x with 300 µL of 1X working Washing Buffer	In each well

100 µL	Substrate Solution **S**	In each well
Incubation: 30 minutes in the dark at 20-25°C		
100 µL	Stop Solution **SL**	In each well
Measure the absorbance within 30 min at **450 nm** with ≥ 590 nm as reference wavelength.		

9. Calculation of Results

For the evaluation of the assay it is required that the absorbance values of the blank should be below 0.25, and the absorbance values of standard E should be above 1.0.
Samples, which yield higher absorbance values than Standard E are beyond the standard curve; for reliable determinations these samples should be re-tested with a higher dilution.

9.1. Establishing the Standard Curve

Standards are provided in the following concentrations:

Standard	**A**	**B**	**C**	**D**	**E**
ng/mL	0.5	2.5	6	12	18

1. Calculate the mean absorbance value for the blank from the duplicate determinations (well A1/A2).
2. Subtract the mean absorbance of the blank from the mean absorbance of all other values.
3. Plot the standard concentrations on the x-axis versus the mean value of the absorbance of the standards on the y-axis.
4. Calculation of the standard curve should be done using a computer program, because the curve is in general (without respective transformation) not ideally described by linear regression. A four-parametric logistic (4-PL) curve fit should be used for recalculation of IGF-I concentrations.
5. The IGF-I concentration of the diluted sample or the diluted control sera KS1&2 in ng/mL (or µg/mL according the chosen unit for the standards) is calculated in this way. The final IGF-I concentration (of the undiluted samples, KS1, and KS2) is calculated by multiplying the value obtained for the diluted samples/controls by their respective dilution factor.

9.2. Example Standard Curve

The following data is for demonstration only and cannot be used in place of data generated at the time of assay.

	Blank	A	B	C	D	E
ng/mL	0	0.5	2.5	6	12	18
OD (450-620 nm)	0.070	0.114	0.614	1.283	1.690	1.923

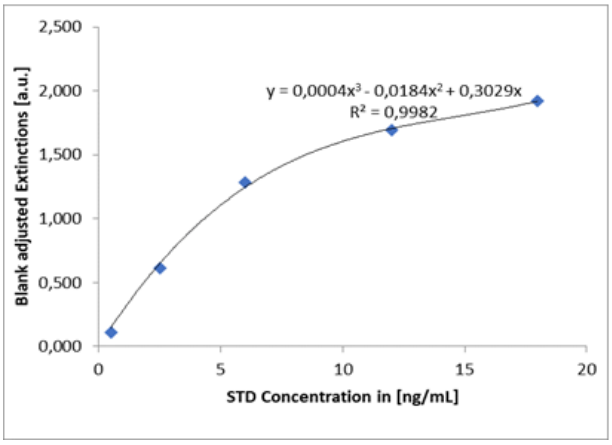

Figure 3: The example standard curve above cannot be used for calculation of your test results. A standard curve must be established for each assay run conducted.

9.3. **Example calculation of IGF-I concentrations**

- Sample dilution: 1:100
- Measured OD of sample: 1.3525
- Measured OD of the blank: 0.07
- The measurement program will calculate the IGF-I concentration of the diluted sample automatically by using the blank subtracted sample value for the calculation.
- In this example, the following equation is solved by the program to calculate the IGF-I concentration:

  $1.283 = \mathbf{0.0004x^3 - 0.0184x^2 + 0.3029x}$

  $6.107 = x$
- If the dilution factor (**1:100**) is considered, the IGF-I concentration of the undiluted sample is 6.107 x100 = 610.7 ng/mL

10. Performance Characteristics

10.1. Analytical Sensitivity

The analytical sensitivity was determined by measuring the blank value and calculating the theoretical concentration of the blank value +2 SD.
The analytical sensitivity of the ELISA is 0.315 ng/mL (range 0.262-0.405 ng/mL).

10.2. Precision

Intra-Assay Variance

Several samples were measured 16 times in the same assay. Example results are shown in Table 1. On average, the coefficient of variation was <10%.

Table 1: The Intra-assay variability. The IGF-I concentrations were determined and variability was calculated as the coefficient of variation (CV).

	Number of Determinations	Mean Value (µg/L)	Standard Deviation (µg/L)	CV (%)
Sample 1	16	246	13.09	5.32
Sample 2	16	684	52.27	7.64
Sample 3	16	679	93.61	13.79

Inter-Assay Variability

Serum samples were measured in independent assays. On average, the coefficient of variation was <10%. Example results are detailed in Table 2.

Table 2: Inter-Assay Variability IGF-I concentrations were determined in independent assays and variability was calculated as the coefficient of variation (CV).

Sample	Number of Determinations	Mean Value (µg/L)	Standard Deviation (µg/L)	CV (%)
Sample 1	24	291	20	7
Sample 2	26	695	51	7
Sample 3	23	773	76	10
Sample 4	23	256	15	6
Sample 5	26	151	21	14
Sample 6	26	444	37	8
Sample 7	26	127	12	9
Sample 8	26	686	59	9
Sample 9	26	581	50	9
Sample 10	26	178	12	7

10.3. Method Comparison

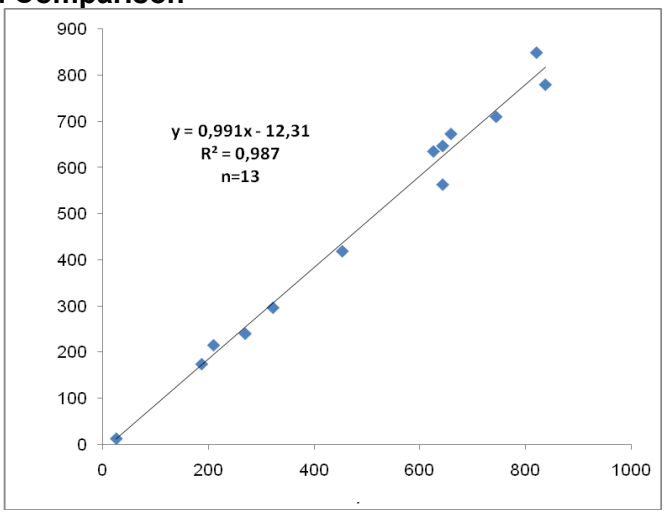

Figure 4: Method comparison of this IGF-I ELISA (y) and another commercially available IGF-I ELISA (x).

10.4. Linearity

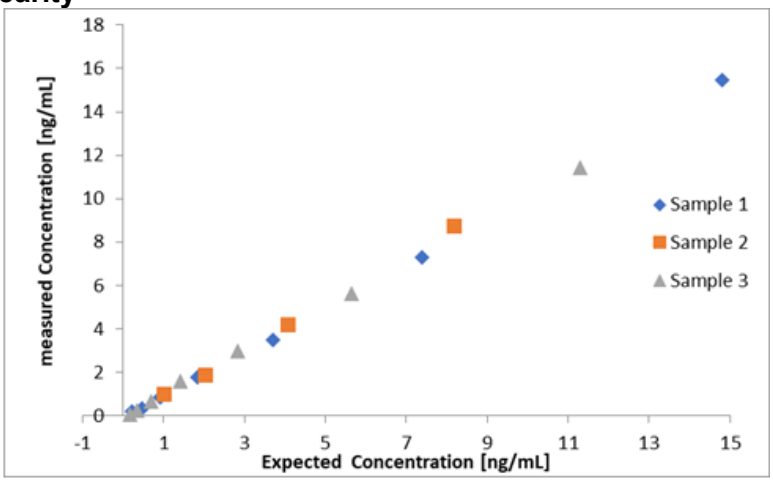

Figure 5: Comparison of the theoretically expected and the measured IGF-I concentrations in the respective dilutions. From a 1:10 dilution to a concentration of 0.5 ng/mL, linearity is demonstrated.

11. References

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